

Effects of Growing Location and Irrigation on Attributes and Ethanol Yields of Selected Grain Sorghums¹

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ABSTRACT

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Nine sorghum cultivars (five inbred lines and four hybrids) were grown in 2006 in three locations (Mount Hope, KS, Halstead, KS, and Plainview, TX) under different irrigation conditions and were evaluated for composition and ethanol fermentation efficiency. The objective was to study, in one growing season, the effects of genotype, growing location, and irrigation on the physical and chemical properties and fermentation efficiencies of grain sorghum. Genotype had a significant effect on chemical composition, physical properties, and ethanol yield. The cultivars showed a large

variation in starch (61.0–74.8%), protein (7.56–16.35%), crude fat (2.79–4.77%), crude fiber (0.58–2.57%), ash (1.25–2.26%), kernel weight (20.0–35.9 mg), kernel hardness (49.6–97.5), and kernel size (1.9–2.7 mm) and were the most important factors affecting ethanol fermentation efficiency (87.5–93.9%). Starch and protein contents were significantly affected by growing location but not by irrigation. Environment had a significant effect on ethanol yields. Unexpectedly, irrigation somewhat reduced fermentation efficiency.

Recently, the ≈20% annual growth in fuel ethanol demand in the United States has resulted in rapid growth in fuel ethanol production capacity, especially across the U.S. Corn Belt. In some areas within the Corn Belt, the concentration of ethanol plants is reaching near-saturation relative to the volume of corn grain available in the vicinity. Opportunities for continued expansion of ethanol production exist in other agricultural regions. One area with high potential for increased contribution is the sorghum production region of the Central Plains. Currently, feedstock for ethanol production is ≈95% corn and 4% sorghum grain (RFA 2007).

Previous research by our group and others has shown that cultivar, amylose-to-amylopectin ratio, protein-starch interaction, the tannin level, mash viscosity, formation of amylose-lipid complex, and particle size of ground sorghum meal had significant effects on ethanol yield and fermentation efficiency from grain sorghum (Zhan et al 2003; Wu et al 2006, 2007). Starch content in grain sorghum was 64–74%, which translates to a difference of 22% in ethanol yield per unit of grain. Ethanol yield from sorghums with similar starch contents varied as much as 7.4%, indicating that not all starch contributes equally to ethanol yield (Wu et al 2007). Heterowaxy and waxy cultivars generally gave higher fermentation efficiencies than other cultivars because these cultivars contain little or no amylose and are less likely to form amylose-lipid complexes. At saccharification and fermentation temperatures, amylose-lipid complex resists enzymatic hydrolysis (Wu et al 2006).

As in other cereal grains, protein content in grain sorghums is inversely proportional to starch content and thus has a negative effect on ethanol yield (Zhan et al 2003; Wu et al 2007). Protein quality also affected ethanol yield by ≤8% for sorghum cultivars with similar protein content. The adverse effects of protein on ethanol fermentation are most likely due to the presence of a cross-

linked protein matrix in sorghum proteins, which can become denser during mashing or cooking. Enmeshed starch granules are less accessible to enzyme hydrolysis. Consequently, digestibility of sorghum starch declines (Zhang and Hamaker 1998; Duodo et al 2003; Wu et al 2007).

Adverse effects of condensed tannins on digestibility of sorghum protein and starch are well known. Wu et al (2007) reported that sorghum tannins retarded the hydrolysis process and resulted in viscous mash that could not be thinned by simply increasing the amylase level during mashing. Efficiency of converting starch to ethanol in 70 sorghum samples was inversely proportional to the viscosity of the mashes.

Several authors (Kelsall and Lyons 2003; Naidu et al 2007) reported that particle size of ground cereals significantly affected ethanol yield from corn and other cereal grains. Ethanol yield from finely ground corn could be 5–10% more than from coarsely ground corn meal. A similar difference in ethanol yields was observed during our study with finely and coarsely ground sorghum (95% < 400 μm vs. 68% < 400 μm).

Growing environments greatly affect physical properties and chemical compositions of cereal grains (Beta and Corke 2001; Tester and Karkalas 2001), which in turn affect their applications (O'Brien and Orth 1977; Mikhaylenko et al 2000; Swanston et al 2005). However, limited research has been conducted to study the effects of growing environment (location and irrigation) on ethanol yields of cereals. Swanston et al (2007) reported that environmental conditions had a significant influence on ethanol yield of wheat cultivars in the United Kingdom. Zhan et al (2003) studied location effects of a few sorghum cultivars in Manhattan, KS, on ethanol yields.

Based on our previous research on ethanol production from >70 sorghum cultivars, we selected nine sorghum cultivars with varying fermentation efficiencies to study the effects of growing location (two locations in Kansas and one in Texas) and irrigation conditions (dryland and irrigated) on physical and chemical properties and fermentation efficiencies.

MATERIALS AND METHODS

Materials

Nine sorghum cultivars (five inbred lines and four hybrids) were planted in three locations: Mount Hope, KS (irrigated and dryland environments), Halstead, KS (dryland only), and Plainview, TX, (irrigated and dryland environments). Cultivar 4211 in irrigated land (TX) was lost because of weather, resulting in a total of 44 grain samples for evaluation. Sorghum grain samples were cleaned by removing debris, dust, and other contaminants. Cleaned samples were ground into fine meals with particle size

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<1.0 mm on a grain mill (III Plus, Magic Mill Products & Appliances, Monsey, NY). Physical properties and chemical compositions of the sorghum samples are listed in Table I.

Potassium phosphate monobasic, magnesium sulfate, dextrose, sodium acetate, hydrochloric acid, sodium hydroxide, and acetic acid were purchased from Fisher Scientific (Fairlawn, NJ). Agar, Difco yeast extract, and Difco peptone were from Becton-Dickinson (Sparks, MD). Maltose, maltotriose, 4-morpholinepropanesulfonic acid (MOPS), and dimethyl sulfoxide (DMSO) were from Supelco (Bellefonte, PA). All chemicals were reagent grade or better.

Hydrolyzing enzymes Liquozyme (high-temperature α -amylase produced by *Bacillus licheniformis*) and Spirizyme (a glucoamylase produced by *Aspergillus niger*) were provided by Novozymes North America (Franklinton, NC). The dry alcohol yeast Ethanol Red was provided by Fermentis in vacuum-packed aluminum foil bags (Lesaffre Yeast Corp., Milwaukee, WI).

Analytical Methods

Crude protein, lipid, and ash were analyzed using AOAC Methods 990.03, 920.39, and 942.05, respectively (AOAC International

1999), and starch was analyzed using Approved Method 76-13 (AACC International 2000). Megazyme total starch assay kits were obtained from Megazyme International Ireland Ltd. (Bray Co., Wicklow, Ireland). Crude fiber was analyzed with the A200 filter bag technique (Ankom Technology, http://www.ankom.com/09_procedures/procedures3.shtml). Ethanol and glucose concentrations were determined by HPLC with a Rezex RCM-mono-saccharide column (300 \times 7.8 mm) (Phenomenex, Torrance, CA) and a reflective index detector (Shimadzu RID-10A, Columbia, MD). The mobile phase was 0.6 mL/min of deionized water and the oven temperature was 80°C (Wu et al 2006).

Physical Properties of Sorghum Kernels

Kernel hardness, kernel size, and thousand-kernel weight were measured using a single-kernel characterization system (SKCS) 4100 (Perten Instruments, Huddinge, Sweden) (Bean et al 2006).

Microstructures of sorghum endosperm were examined using a Hitachi S-3500N scanning electron microscope (SEM) with an S-6542 absorbed electron detector (Hitachinaka, Ibaraki, Japan). Samples were coated with 4 nm of a 60% gold and 40% palladium mixture in a Denton vacuum chamber (Desk II, Moores-

TABLE I
Chemical Composition (%db) and Physical Properties of Sorghum Cultivars

| Samples | Starch (%) | Protein (%) | Fat (%) | Fiber (%) | Ash (%) | Hardness Index | Kernel Wt (mg) | Kernel Size (mm) |
|----------------------------|------------|-------------|---------|-----------|---------|----------------|----------------|------------------|
| Mt. Hope, KS | | | | | | | | |
| 4175 dryland | 64.4 | 14.81 | 3.80 | 1.50 | 1.98 | 78.4 | 27.9 | 2.3 |
| 4175 irrigated | 66.3 | 14.45 | 3.85 | 1.59 | 1.90 | 82.5 | 26.4 | 2.2 |
| 4224 dryland | 66.5 | 10.51 | 3.72 | 1.26 | 1.66 | 75.0 | 25.3 | 2.2 |
| 4224 irrigated | 67.4 | 11.35 | 3.77 | 1.42 | 1.72 | 84.2 | 25.5 | 2.3 |
| 4174 dryland | 69.2 | 11.50 | 3.79 | 1.03 | 1.81 | 78.6 | 21.2 | 2.3 |
| 4174 irrigated | 68.0 | 13.45 | 3.80 | 1.19 | 1.75 | 95.7 | 20.7 | 2.3 |
| 4162 dryland | 74.8 | 7.56 | 2.80 | 0.61 | 1.25 | 76.7 | 30.6 | 2.5 |
| 4162 irrigated | 74.2 | 9.00 | 2.79 | 1.39 | 1.31 | 78.4 | 28.7 | 2.5 |
| 4168 dryland | 70.0 | 10.02 | 3.44 | 0.67 | 1.64 | 76.2 | 27.2 | 2.3 |
| 4168 irrigated | 70.5 | 10.52 | 3.25 | 1.52 | 1.53 | 74.9 | 27.7 | 2.4 |
| 4159 dryland | 71.3 | 10.08 | 2.96 | 0.58 | 1.42 | 73.6 | 30.6 | 2.4 |
| 4159 irrigated | 72.5 | 9.80 | 2.91 | 1.36 | 1.34 | 77.9 | 28.9 | 2.4 |
| 4161 dryland | 70.6 | 9.68 | 3.36 | 1.46 | 1.47 | 66.9 | 30.2 | 2.3 |
| 4161 irrigated | 72.0 | 10.05 | 3.30 | 1.64 | 1.33 | 78.7 | 32.4 | 2.4 |
| 4209 dryland | 67.8 | 11.22 | 3.35 | 1.51 | 1.67 | 49.6 | 26.1 | 2.0 |
| 4209 irrigated | 67.2 | 12.63 | 3.18 | 1.56 | 1.59 | 64.6 | 26.6 | 2.1 |
| 4211 dryland | 70.1 | 9.95 | 3.00 | 1.33 | 1.57 | 61.8 | 23.4 | 2.1 |
| 4211 irrigated | 67.4 | 12.88 | 3.30 | 1.52 | 1.77 | 71.0 | 28.8 | 2.4 |
| Plainview, TX | | | | | | | | |
| 4175 dryland | 61.0 | 16.35 | 4.61 | 2.14 | 2.26 | 95.2 | 27.9 | 2.3 |
| 4175 irrigated | 61.6 | 16.31 | 4.77 | 2.57 | 2.15 | 97.5 | 28.6 | 2.3 |
| 4224 dryland | 63.6 | 13.17 | 4.03 | 2.09 | 2.11 | 84.9 | 24.6 | 2.1 |
| 4224 irrigated | 64.7 | 13.08 | 4.08 | 2.35 | 2.04 | 81.3 | 26.3 | 2.2 |
| 4174 dryland | 65.7 | 15.18 | 3.85 | 1.60 | 1.93 | 96.6 | 20.0 | 2.2 |
| 4174 irrigated | 66.1 | 14.80 | 4.04 | 1.97 | 1.91 | 94.2 | 20.8 | 2.3 |
| 4162 dryland | 67.7 | 13.01 | 2.86 | 1.85 | 1.61 | 77.4 | 32.8 | 2.6 |
| 4162 irrigated | 70.0 | 12.11 | 3.05 | 2.15 | 1.56 | 84.1 | 30.6 | 2.6 |
| 4168 dryland | 66.0 | 13.96 | 3.62 | 1.89 | 1.78 | 94.9 | 30.8 | 2.5 |
| 4168 irrigated | 65.9 | 15.04 | 3.39 | 2.10 | 1.97 | 87.2 | 31.3 | 2.5 |
| 4159 dryland | 68.3 | 12.98 | 3.25 | 1.66 | 1.64 | 81.1 | 31.6 | 2.4 |
| 4159 irrigated | 71.1 | 11.05 | 3.26 | 1.95 | 1.47 | 75.8 | 32.2 | 2.5 |
| 4161 dryland | 67.5 | 12.77 | 3.44 | 1.90 | 1.62 | 81.1 | 34.5 | 2.6 |
| 4161 irrigated | 69.8 | 11.63 | 3.44 | 2.06 | 1.55 | 75.8 | 35.9 | 2.6 |
| 4209 dryland | 67.6 | 12.01 | 3.44 | 1.68 | 1.84 | 67.5 | 24.3 | 2.0 |
| 4209 irrigated | 66.7 | 12.82 | 3.46 | 1.93 | 1.87 | 70.4 | 23.4 | 1.9 |
| 4211 dryland | 67.8 | — | 3.17 | 1.79 | 1.59 | 87.7 | 25.7 | 2.2 |
| 4211 irrigated | — | — | — | — | — | — | — | — |
| Halstead, KS (all dryland) | | | | | | | | |
| 4175 | 64.0 | 14.63 | 4.13 | 1.84 | 2.03 | 84.6 | 30.5 | 2.3 |
| 4224 | 64.3 | 12.23 | 3.69 | 2.12 | 1.92 | 79.7 | 25.1 | 2.2 |
| 4174 | 65.3 | 14.68 | 3.74 | 1.60 | 2.01 | 76.3 | 21.4 | 2.3 |
| 4162 | 74.0 | 8.79 | 2.88 | 1.65 | 1.42 | 77.7 | 32.6 | 2.7 |
| 4168 | 69.9 | 10.79 | 3.46 | 1.65 | 1.64 | 76.7 | 29.0 | 2.5 |
| 4159 | 71.6 | 11.02 | 3.06 | 1.50 | 1.43 | 77.9 | 35.8 | 2.6 |
| 4161 | 69.3 | 11.89 | 3.31 | 1.64 | 1.54 | 74.1 | 33.3 | 2.5 |
| 4209 | 64.5 | 13.54 | 3.40 | 1.93 | 1.91 | 52.9 | 24.2 | 1.9 |
| 4211 | 66.0 | 13.71 | 3.11 | 1.73 | 1.76 | 66.9 | 27.6 | 2.3 |

town, NJ) operating at 100 milli-torr for ≈ 45 sec before SEM examination. Images were taken of both whole and representative cross-sections of enlarged endosperm parts 1–1.5 mm away from the kernel edge.

Differential scanning calorimetry (DSC) analysis of sorghum meals was performed with a Pyris 1 differential scanning calorimeter (Perkin Elmer Corp., Norwalk, CT) equipped with the Pyris 1 Data Analysis System for Windows. Samples (≈ 10 mg) were weighed in a stainless steel pan on a CAHN 21 automatic electrobalance (Cerritos, CA) and mixed with distilled water (≈ 30 μ L) to produce mixtures of $\approx 75\%$ moisture content. Sealed samples were kept at 4°C overnight before being tested. A sealed, empty stainless steel pan was used as reference. All samples were held at 20°C for 1 min and heated from 20 to 160°C at 10°C/min.

Ethanol Fermentation

Ground samples containing 30.00 g, dry mass, were mixed with 100 mL of preheated (≈ 60 – 70°C) enzyme solution containing (g/L) 1.0 g of KH_2PO_4 and 200 μ L of Liquezyme in a clean, 250-mL Erlenmeyer flask to form an evenly suspended slurry. Flasks were kept at 70°C in a rotary water bath shaker operating at ≈ 180 rpm. Temperature of the water bath was raised from 70 to 90°C in 35–40 min, kept at 90°C for a few minutes, and then lowered to 85°C; liquefaction continued for 60 min. Material sticking on the inner surface of the flasks was pushed back into the mashers with a spatula. The spatula and inner surface of the flasks were rinsed with 3–5 mL of distilled water. After cooling to room temperature (≈ 25 – 30°C), the mashers were adjusted to pH ≈ 4.2 with 2N HCl.

Before inoculation, dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extracts, 1.0 g of KH_2PO_4 , and 0.5 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ /L) and incubated at 38°C for 25–30 min in an incubator operating at 200 rpm. The activated yeast culture had a cell concentration of $\approx 1 \times 10^9$ cells/mL.

The simultaneous saccharification and fermentation (SSF) process started with the addition of 1.0 mL of activated yeast culture, 100 μ L of Spirizyme (750 AGU/g, ≈ 1.15 g/mL), and 0.30 g of yeast extract to mashers in each flask. Flasks were sealed with an S-airlock filled with mineral oil. Fermentation was conducted at 30°C for 72 hr in an incubator shaker operating at 150 rpm. The fermentation process was monitored by measuring weight loss of the fermenting mash due to evolution of CO_2 during fermentation.

Ethanol concentration in the finished beer was determined by HPLC after distillation as described by Wu et al (2006). The conversion efficiency was calculated from the theoretical yield of 56.72 g of ethanol produced from 100.0 g of dry starch.

Statistical Analysis

Effects of location and irrigation on starch and protein contents and fermentation efficiency were analyzed using PROC MIXED. Differences among lines for each trait at the same location were determined using the LSD LINE option of PROC GLM. Simple correlations of physical and chemical traits were determined using PROC CORR (SAS Institute, Cary NC).

RESULTS AND DISCUSSION

Geographic location and irrigation had significant effects on physical attributes, chemical compositions, and ethanol yields of the sorghum cultivars. Starch, protein, crude fat, and ash contents were significantly affected by geographic location; crude fiber content and kernel hardness were significantly affected by both geographic location and irrigation; fermentation efficiency was affected only by irrigation and not by geographic location.

Effects on Chemical Composition and Physical Properties of Sorghum Samples

Sorghum cultivars growing in Kansas (Mount Hope and Halstead) generally had significantly ($P < 0.0001$) higher starch contents (average of 69.5% for Mount Hope and 67.7% for Halstead samples) than cultivars (average of 66.5%) in Texas (Plainview) (Fig. 1). Starch contents of sorghums grown in Texas were 3.3–7.1% lower than those from Kansas, indicating that the same sorghum cultivar would have ≈ 5 – 10% less starch content if planted in Texas instead of Kansas. The cooler climate in Kansas during the grain-filling period of late August to September might have given the Kansas sorghums a longer grain-filling period than occurred in Texas during June and July. Longer grain-filling period results in an increase in starch deposition relative to nitrogen taken up and leads to higher starch content and lower protein content in Kansas sorghums. Sorghum cultivars with high starch and low protein content are cultivars of choice for fuel ethanol production. Higher starch content means higher ethanol yield, better processing efficiency, and less leftover residues after fermentation.

In contrast to starch, sorghums grown in Texas had significantly higher contents of protein, crude fat, crude fiber, and ash (average of 13.52, 3.64, 1.98, and 1.82%, respectively) (Fig. 2) than sorghums grown in Kansas (average of 11.08, 3.37, 1.29, and 1.60%, respectively, for Mount Hope samples and 12.36, 3.42, 1.74, and 1.74%, respectively, for Halstead samples). Protein contents of sorghum grown in Texas were 0.79–5.45% higher than the counterparts in Kansas, which translates to a 7–72% difference in protein content due to the location and environment (Table I). Low digestibility of sorghum protein and its adverse effects on starch

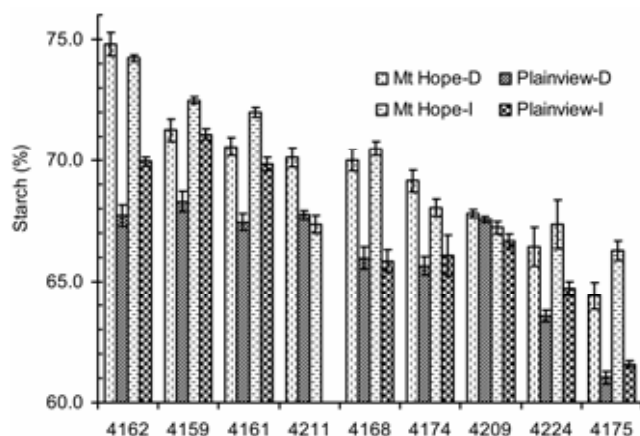


Fig. 1. Comparison of starch contents among 35 samples from different growing locations. Error bars are standard deviation of four determinations.

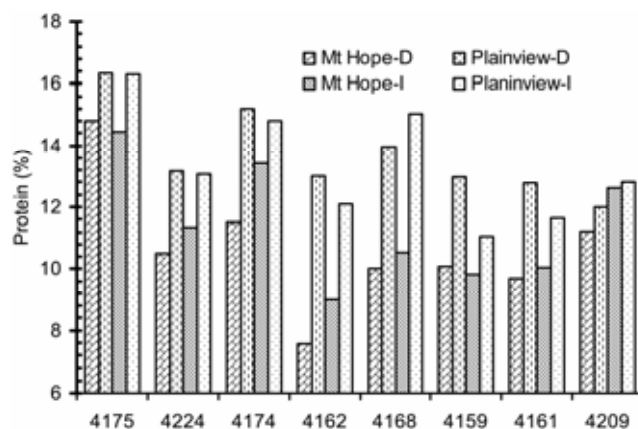


Fig. 2. Protein contents (means of two analyses) among 32 samples from different growing conditions.

digestion are thought to be related to the cross-linking of sorghum proteins during cooking and the formation of a web-like protein matrix that inhibits the approach of hydrolyzing enzymes to the starch inside the protein matrix (Wu et al 2007). Results from the present study revealed a similar trend; sorghums with higher protein contents, such as samples 4174 and 4175, had lower ethanol fermentation efficiencies than other samples across all locations and under both irrigation conditions (Fig. 3). Representative SEM images (Fig. 4) of endosperm cross-sections of sorghum kernels from both Kansas and Texas clearly showed differences in cell wall structure and protein bodies between Kansas and Texas sorghums. Cell wall structures in Texas sorghums were thicker and intact, but those in Kansas sorghums were thin, fragile, and broken. The images also showed that there were more protein bodies on the surface of starch granules in the irrigated samples than in the dryland samples, which could partially explain the lower efficiency of irrigated samples compared with dryland samples. Significant improvement in starch yield during wet milling of sorghum by the addition of cell-wall-degrading enzymes and proteases also demonstrated the protection effects of cell wall and protein matrix on starch granules (Perez-Carrillo and Serna-Saldivar 2006). Specific processing such as supercritical-fluid-extrusion cooking also might destroy the protein matrix in grain sorghum and significantly improve ethanol yield (Zhan et al 2006).

Shi et al (1994) and Tester and Karkalas (2001) reported that growing temperature could significantly affect the lipid contents

of starches from rice, barley, and wheat. Starches from cereals grown in a high-temperature environment had elevated gelatinization temperatures and increased lipid contents in their starches. This could not explain the observations of DSC thermograms (Fig. 5) in our study. Sorghum samples from Mount Hope, KS, showed higher starch gelatinization temperatures (1–3°C higher) and increased proportion of the amylose-lipid peaks in thermograms compared with samples from Plainview, TX, indicating that starches from Kansas sorghums were somewhat more difficult to gelatinize than those from Texas sorghums. However, ethanol fermentation results showed that growing location did not have significant effects on conversion efficiency, demonstrating that any adverse effects of the slightly elevated gelatinizing temperature of starch and an increase in amylose-lipid complex in Kansas sorghums were not sufficient to generate a significant difference in fermentation efficiency within a genetic line. Furthermore, across different cultivars, the amylose-lipid complex peak could be a very good indicator of fermentation efficiency. High-efficiency cultivars (4224, 4209, and 4162) had no or a relatively small amylose-lipid complex peak in DSC thermograms, but low-efficiency cultivars (4174 and 4175) did have significantly larger amylose-lipid complex peaks (Fig. 5).

Kernel hardness is closely related to protein content (Table II) and both could play a role in affecting fermentation efficiency of sorghum. Typically, the higher the protein content, the harder the sorghum grain kernel and the lower the accessibility of hydrolyzing enzymes to starch in the ground meal during mashing and fermentation processes. Location had a similar effect on hardness index and protein content of sorghums. Sorghums from Texas had significantly higher kernel hardness (average hardness index of 84.3) than those from Kansas (average hardness indexes of 74.7 and 74.1 for the samples from Mount Hope and Halstead, respectively).

In addition to location effects, irrigation also had a significant effect on kernel hardness. Kernels from irrigated land had a significantly higher hardness index than those from dryland. The inverse relationship between kernel hardness and fermentation efficiency is in agreement with the effects of irrigation on fermentation effects. Correlations between other parameters are listed in Table II. No significant interaction between location and irrigation was found for starch and protein contents of sorghum samples.

Effects on Fermentation Efficiency and Ethanol Yield

Although there was no significant ($P = 0.0839$) difference in efficiency across the three locations, effects of irrigation on fermentation efficiency were significant ($P = 0.0045$) (Fig. 6).

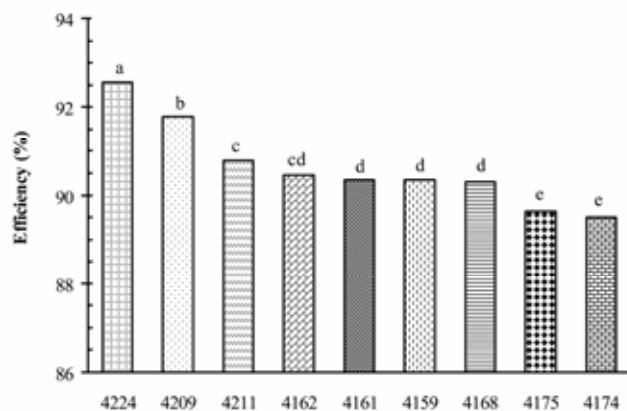


Fig. 3. Comparison of fermentation efficiencies among 44 samples of nine sorghum cultivars. Bars with different letters are significantly different in efficiency ($P < 0.05$).

TABLE II
Pearson Correlation Coefficients with Probability Value Among Evaluated Parameters for 44 Samples

| | Hardness Index | Kernel Weight | Kernel Size | Crude Fat | Crude Fiber | Ash | Protein | Starch | Efficiency |
|----------------|----------------------|-------------------------|----------------------|--------------------------|--------------------------|--------------------------|---------------------------|----------------------|------------|
| Hardness Index | 1.0000 | | | | | | | | |
| Kernel Weight | -0.04253 (0.7840) | 1.0000 | | | | | | | |
| Kernel Size | 0.3436 (0.0224) | 0.7329 (<0.0001) | 1.0000 | | | | | | |
| Crude Fat | 0.5182 (0.0003) | -0.3867 (0.0095) | -0.3354 (0.0260) | 1.0000 | | | | | |
| Crude Fiber | 0.2757 (0.0701) | 0.09996 (0.5185) | -0.03958 (0.7986) | 0.4498 (0.0022) | 1.0000 | | | | |
| Ash | 0.3379 (0.0249) | -0.4809 (0.0010) | -0.4701 (0.0013) | 0.8492 (<0.0001) | 0.5364 (0.0002) | 1.0000 | | | |
| Protein | 0.4593 (0.0019) | -0.2781 (0.0710) | -0.2480 (0.1089) | 0.7217 (<0.0001) | 0.6074 (<0.0001) | 0.8665 (<0.0001) | 1.0000 | | |
| Starch | -0.2837 (0.0620) | 0.3881 (0.0092) | 0.4870 (0.0008) | -0.8079 (<0.0001) | -0.5843 (<0.0001) | -0.9415 (<0.0001) | -0.88743 (<0.0001) | 1.0000 | |
| Efficiency | -0.3951 (0.0080) | -0.1512 (0.3273) | -0.4182 (0.0047) | -0.06196 (0.6895) | -0.0748 (0.6294) | -0.03691 (0.8120) | -0.32301 (0.0346) | -0.08177 (0.5977) | 1.0000 |

Eight of the nine samples from dryland had significantly higher fermentation efficiency than samples from irrigated land. Fermentation efficiency of samples from dryland in Mount Hope, KS, was 0.3–2.7% (average of 1.03%) higher than equivalent samples from irrigated land. This result might be related to starch-protein interactions in the endosperm. For example, the SEM images (Fig. 4) show that starch granules in grain from dryland condi-

tions usually have smooth surfaces and few protein bodies around individual starch granules, but starch granules in grain from irrigated land have various degrees of association with protein bodies.

These protein bodies can cross-link and enmesh starch granules during mashing and, therefore, block this fraction of starch from exposure to hydrolyzing enzymes, resulting in lower fermentation

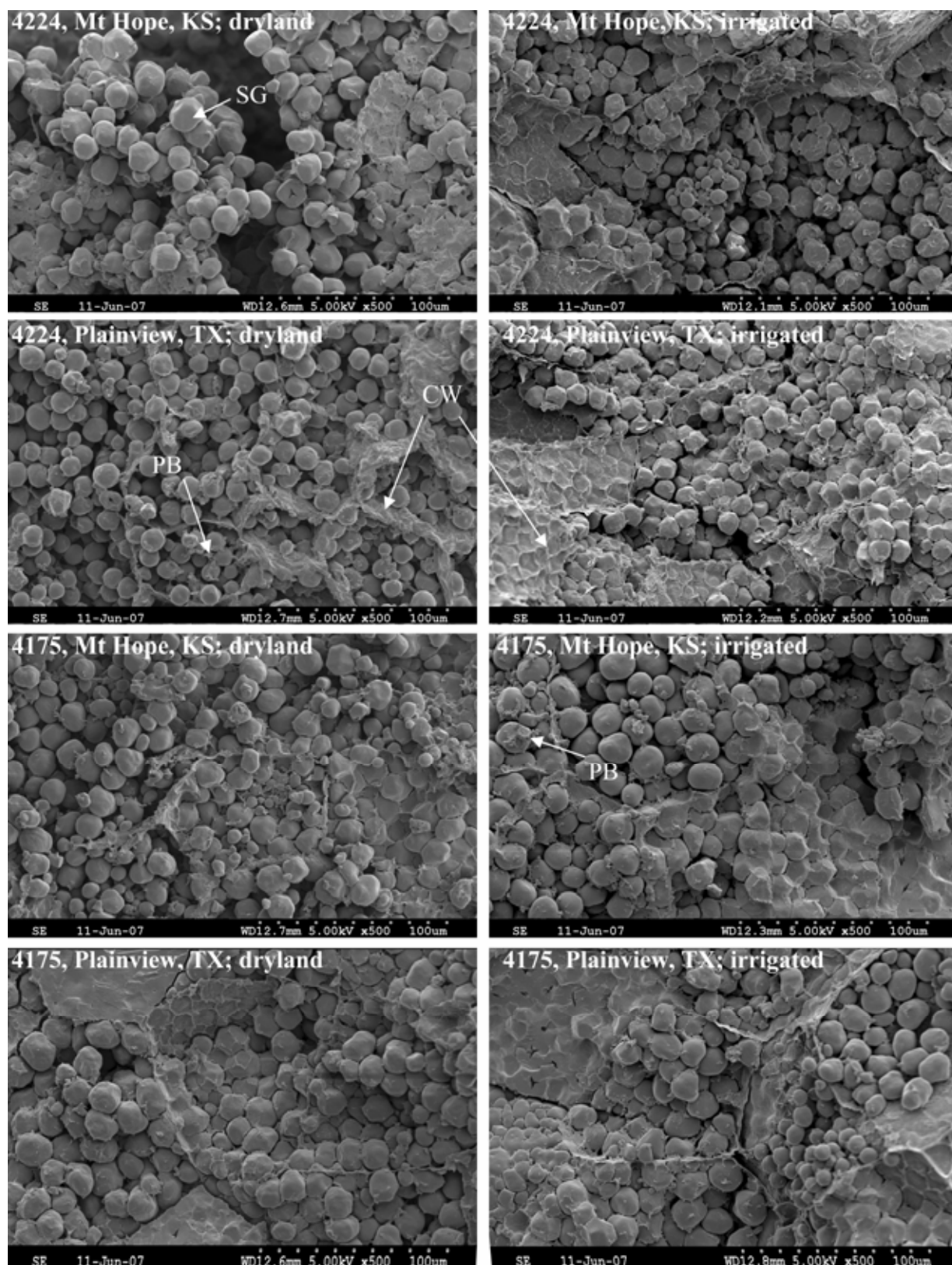


Fig. 4. SEM images of starch granules and protein matrix in sorghum endosperm (SG, starch granule; CW, cell wall; PB, protein body).

efficiency. More research is needed to investigate the function and fate of these protein bodies and elucidate why dryland sorghums had better fermentation efficiency than irrigated sorghums.

Among all factors, genetic line was the most significant factor ($P < 0.0001$) affecting fermentation efficiency across all locations and irrigating conditions. The waxy cultivar performed better, in terms of efficiency, during ethanol fermentation than other cultivars in all locations and under both irrigation conditions (Fig. 3). This finding agreed with results of previous work (Wu et al 2006, 2007). Low gelatinization temperature, higher swelling capacity of waxy starch, and the little or no amylose-lipid complex in waxy sorghum all might play a role in the higher digestibility during enzymatic hydrolysis and higher fermentation efficiency of waxy sorghum.

Sorghum samples 4174 and 4175 had the lowest fermentation efficiencies and lowest ethanol yields of all the samples (Fig. 7). The DSC thermal analysis of ground sorghum grain revealed that the amylose-lipid portion was larger in cultivars 4174 and 4175 than in any of the other samples (data not shown). The correlation between fermentation efficiency and the percentage of amylose-lipid peak in the total starch peak had an R^2 of 0.53 in the DSC thermograms, with $y = -9.25x + 92.44$, where y is the fermentation efficiency and x is the percentage of amylose-lipid peak.

Considering both conversion efficiency and ethanol yield, the ideal sorghum feedstock for the commercial ethanol industry

would have high grain yield per acre combined with high starch content and low levels of lipids and amylose-lipid complexes. Currently, any one cultivar seldom possesses all desirable traits. Improvements through breeding can bring more favorable traits into one genetic line; however, achieving this on a routine basis remains a challenge. As shown in this study, the difference in fermentation efficiency among sorghum cultivars due to growing location was 0.3–2.7%, much smaller than the 13% difference in starch content caused by growing location. Cultivars 4224 (waxy) and 4209 (normal) had significantly higher fermentation efficiencies (average of 92.6 and 91.8%, respectively) than the other cultivars (Fig. 3), but the starch contents (average of 65.3 and 66.8%, respectively) were significantly lower than most other cultivars (Fig. 1). However, if the starch contents of cultivars 4224 and 4209 cannot be increased by $\approx 5\%$ through breeding while maintaining high fermentation efficiencies, then sorghum 4162 would be a better choice for the ethanol industry because of its high starch content (average of 72.1%) and moderately high ethanol fermentation efficiency (average of 90.5%), which leads to a higher ethanol yield than hybrids 4224 and 4209 (Fig. 7).

CONCLUSIONS

Sorghum cultivar was the most important factor affecting fermentation efficiency and ethanol yield in a simulated dry-grind process. In one growing year in Texas and Kansas, growing location had significant effects on grain physical properties, chemical composition, and ethanol yield but did not have significant effects on fermentation efficiency. In commercial practice, some variation in ethanol output might be encountered with feedstock from different locations, even with the same genetics, but the degree of variability will be lower than that found across germplasm. Irrigation significantly affected fermentation efficiency, kernel hardness, and crude fiber content of the tested cultivars. Across genetic lines and test conditions used in this study and for the parameters measured, there was no genotype-environment interaction.

The waxy cultivar gave the highest fermentation efficiency, regardless of growing location and irrigation conditions. A waxy phenotype combined with high starch content might be the ideal grain feedstock for ethanol production. High starch is a starting point for increased ethanol yield, but genetics and environment can affect ethanol output because of changes in the starch-protein matrix in the grain. Ensuring that the protein matrix does not restrict accessibility of starch to the hydrolytic enzymes is important. Further research is required to determine the characteristics of an "optimum" matrix and how that can be best achieved in practice.

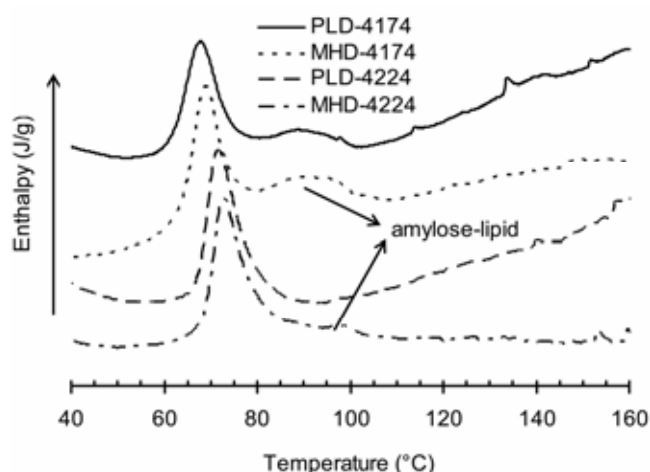


Fig. 5. Differential scanning calorimetry thermograms of sorghum flours from different growing locations and irrigation conditions (PLD, dryland, Plainview, TX; MHD, dryland, Mount Hope, KS).

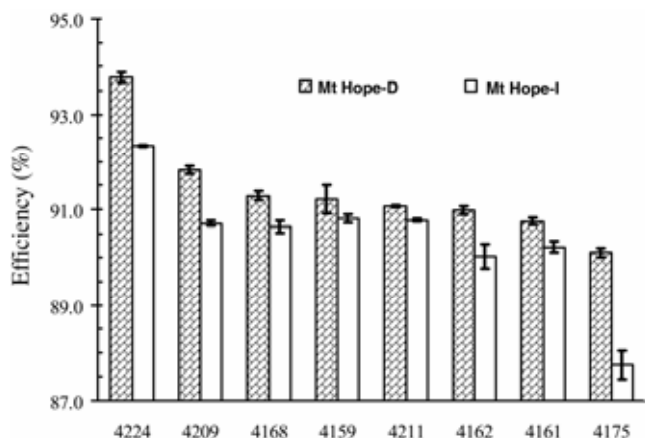


Fig. 6. Fermentation efficiency of 16 sorghum samples from Mount Hope, KS (dryland and irrigated). Error bars are standard deviations of four determinations.

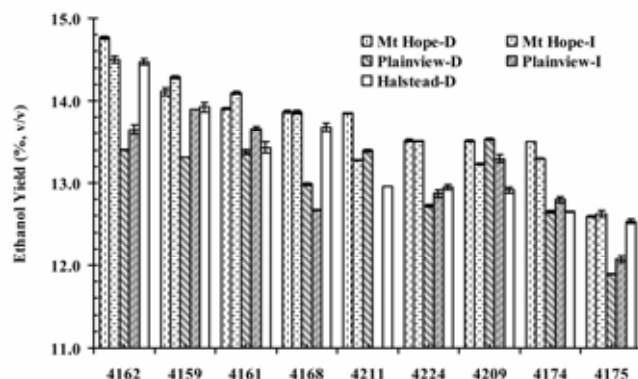


Fig. 7. Ethanol yields of 44 samples of nine sorghum cultivars from different growing locations and irrigation conditions. Error bars are standard deviation of four determinations.

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